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RESEARCH ARTICLE

Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Predicting Worsening Renal Function in Acute and Chronic Heart Failure

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Abstract

Background: The development of worsening renal function (WRF, defined as serum creatinine rise ≥0.3 mg/dL) occurs frequently in the setting of acute decompensated heart failure (ADHF) and strongly predicts adverse clinical outcomes. NGAL has emerged as promising and sensitive biomarker of early AKI in a diverse range of settings, as NGAL rapidly appears in both blood and urine in response to renal tubular damage. Renal insufficiency as assessed by a reduction in estimated glomerular filtration rate (eGFR) is frequently observed in patients with chronic heart failure(CHF) and is associated with reduced survival**.** **Objective:** To assess the role of serum NGAL in predicting subsequent development of WRF in case of ADHF and as a diagnostic marker for renal impairment in CHF and to establish the relationship between serum NGAL and other renal parameters (BUN, creatinine and eGFR). **Methods:** Our study included 30 patients with chronic HF (group I), 30 patients with acute HF (group II) and 20 subjects; age and sex matched apparently healthy individuals as controls (groupIII). All subjects gave informed written consent to participate in the study. All subjects were subjected to full history taking, complete clinical examination, chest x-ray, ECG, Echocardiography and laboratory investigations including CBC, BUN and serum creatinine, eGFR and serum NGAL level. In acute HF group; samples for NGAL assay were obtained at admission and on 3rd day, while changes in BUN, serum creatinine and eGFR were monitored during their hospital stay for 3 days to assess WRF. **Results:** BUN, serum creatinine and NGAL levels were significantly higher, while eGFR values were significantly lower in chronic HF cases as compared with their controls. A ROC curve analysis was performed to evaluate the individual diagnostic values of serum NGAL, creatinine and BUN for renal impairment in chronic HF cases. An optimal NGAL cutoff at >18µg/L, yielding 93% sensitivity and 76% specificity with area under curve (AUC) of 0.912. While BUN and serum creatinine had AUC of 0.625 and 0.729 respectively**.** In acute HF group**,** BUN, Serum creatinine and NGAL levels on admission were significantly higher, while, eGFR values were significantly lower in acute HF cases as compared with their controls**.** Also, BUN, serum creatinine and NGAL levels were significantly higher, while eGFR values were significantly lower in acute HF cases with WRF than their levels in cases without WRF at admission and on 3rd day. A ROC curve analysis was performed to evaluate predictive values of admission NGAL, creatinine and BUN for WRF in acute HF. An optimal admission NGAL cutoff at > 27.5µg/L yielding 90% sensitivity and 68% specificity with AUC 0.969. While BUN and serum creatinine had AUC of 0.569 and 0.684 respectively. **Conclusion:** NGAL can be considered as a sensitive diagnostic marker superior to both BUN and creatinine for early detection of impaired renal function in HF even before eGFR is markedly reduced.

Introduction

Heart failure occurs when the heart cannot deliver adequate cardiac output to meet the metabolic needs of the body. In the early stages of heart failure, various compensatory mechanisms are evoked to maintain normal metabolic function. When these mechanisms become ineffective, several clinical manifestations result[(1)].

Both the heart and the kidney act in tandem to regulate blood pressure, vascular tone, diuresis and natriuresis, and this is called cardiorenal connection[(2)].

The development of worsening renal function (WRF, defined as an increase in serum creatinine (S.Cr) ≥0.3 mg/dL (≥26.5 μmol/L) or ≥25% relative to S.Cr at the time of hospital admission), occurs frequently in the setting of acute decompensated heart failure (ADHF) and strongly predicts adverse clinical outcomes[(3)].

A little is known regarding the pathophysiology of the cardiorenal syndrome (CRS). A reduced cardiac output in congestive heart failure resulting in decreased renal perfusion could be an easy explanation for WRF. Interestingly, WRF has been demonstrated in patients with ADHF even though left ventricular ejection fraction (EF) is preserved[(4)]. This decline in renal function, despite a presumed preservation of blood flow to the kidneys, has led to the search for other mechanisms of CRS, including the role of the renin-angiotensin-aldosterone system, various chemicals (nitric oxide, prostaglandins, natriuretic peptides, endothelins, etc), oxidative stress and sympathetic overactivity[(4)].

The current most widely used biomarkers for the early detection of chronic kidney disease (CKD) or acute kidney injury (AKI) are proteinuria, serum creatinine, and blood urea nitrogen (BUN). All of these are less than optimal and tend to focus attention on later stages of injury when therapies may be less effective[(5)].

Serum creatinine rise occurs when a significant amount of renal function has been lost. Many factors are able to modify its physiological levels, such as age, gender, ethnicity, dietary protein intake, muscle mass or metabolism, hydration status and drugs[(6)].

Since early treatment is associated with improved clinical results, it is thus essential to identify new biomarkers with substantial predictive power to reduce the serious sequelae . Indeed, considerable efforts and progress have been made over the last few years in the search for novel biomarkers[(7)].

There has been a great surge of interest in identifying novel biomarkers that can be easily detected in urine that can diagnose renal injury at the earliest stages. Currently, several candidate biomarkers have been identified and studied in different renal injury states. These include neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin 18 (IL-18), and fatty-acid binding proteins (FABPs)[(5)].

The small 25 kDa peptide, neutrophil gelatinase-associated lipocalin (NGAL), is first known as an antibacterial factor of natural immunity, and an acute phase protein[(8)].

Due to the lack of sensitive and specific biomarkers, the identification of early stages of renal injury has been impossible but, NGAL is emerging as a novel biomarker of renal injury from several etiologies[(9)]. .

We aim to assess the role of serum neutrophil gelatinase-associated lipocalin in predicting subsequent development of worsening renal function (WRF) in the case of acute and chronic heart failure.

SUBJECTS AND METHODS

This observational cohort study was carried out at Internal Medicine and Clinical Pathology Departments, Faculty of Medicine, Zagazig University during the period from July 2013 to July 2014.

Eighty subjects, whom were randomly selected and were included in this study. They were divided into three groups

Group I (patients with chronic heart failure):

It included 30 patients (14 males and 16 females) with mean age ±SD of 48.5 ±10.1 years. These patients had chronic heart involvement in the form of left ventricular ejection fraction (LVEF) <45%.

Group II (patients with acute heart failure):

It included 30 patients (15 males and 15 females) with mean age ±SD of 47.9 ± 11 years. These patients had acute heart involvement in the form of shortness of breath, tachycardia and tachypnea.

Group III (Control group):

It included 20 age and sex matched apparently healthy volunteers (9 males and 11 females) with mean age ± SD of 48.2 ± 10 years with no history of cardiac or renal problems.

Exclusion criteria:

1-Patients with end-stage renal disease (ESRD) on dialysis.

2- Known exposure to nephrotoxic agents (i.e. contrast dye).

3- History of recurrent urinary tract infection (UTI).

Methods:

Review of system information and the results of routine laboratory testing at the time of the study visits were recorded. Relevant demographic data of all participants were obtained. All subjects gave informed written consent to participate in the study, which was approved by the ethics review committee of faculty of medicine, Zagazig University.

All individuals included in this study were subjected to:

A- Full history taking.

B- Thorough clinical examination.

C-Assessment of severity of heart failure according to New York Heart Association(NYHA) functional classification:

Class I: No limitation is experienced in any activities; there are no symptoms from ordinary activities.

Class II: Slight, mild limitation of activity; the patient is comfortable at rest or with mild exertion.

Class III: Marked limitation of any activity; the patient is comfortable only at rest.

Class IV: Any physical activity brings on discomfort and symptoms occur at rest[(10)].

D- X-ray chest &heart.

E- Standard 12 Lead Electrocardiography (ECG)

F- Echocardiography evaluation.

G- Routine laboratory investigations:

1- Complete blood count (CBC) was done using Sysmex KX 21 N hematology analyzer.

2- Renal function tests (BUN and serum creatinine) were done using Dimension RXL autoanalyzer (Siemens Diagnostics).

3- Calculation of glomerular filtration rate using the Modification of Diet in Renal Disease Study(MDRD) equation:

eGFR (mL/min/1.73 m2) = **175** x $(Scr)^{-1.154}$ x $(Age)^{-0.203}$ x (0.742 if female).

H- Quantitative measurement of serum NGAL **(**neutrophil gelatinase-associated lipocalin) was done by enzyme-linked immunosorbent assay (ELISA) using Lipocalin-2 / NGAL ELISA Kit , Cat. No. 036 , Bio Porto Diagnostics , Denmark

- Principle of assay :

Sandwich ELISA performed in microwells coated with a monoclonal antibody to human NGAL. Bound NGAL is detected with another monoclonal NGAL antibody labeled with biotin and the assay is developed with horseradish peroxidase (HRP)-conjugated streptavidin followed by the addition of a substrate to form a color.

- **Collection of samples :**

Venous blood was withdrawn under aseptic conditions in plain tubes , after clotting of blood the sample was centrifuged , serum was separated and stored at – 20 C° until the time of assay.

- **Procedure** :

▪ Serum samples were diluted 1 : 500 in sample diluent before assay

▪ 100 ul of calibrators and diluted samples were incubated in microwells precoated with monoclonal capture antibody for 60 minutes at room temperature. NGAL present in the solutions could bind to the coat , while unbound material was removed by washing.

▪ 100 ul of biotinylated monoclonal detection antibody was added to each test well and incubated for 60 minutes at room temperature which allowed the detection antibody to attach to bound NGAL , while unbound detection antibody was removed by washing.

▪ 100 ul of HRP-conjugated streptavidin was added to each test well and allowed to form a complex with the bound biotinylated antibody during incubation for 60 minutes at room temperature. Unbound conjugate was removed by washing.

▪ 100 ul of color-forming peroxidase substrate containing tetramethylbenzidine (TMB) was added to each test well and incubated for exactly 10 minutes at room temperature in the dark. The bound HRP-streptavidin reacted with the substrate forming a colored product.

▪ 100 ul of Stop solution was added to each well to stop the enzymatic reaction.

6- The absorbance of wells was read within 30 minutes at 450 nm wavelength in a microplate reader (reference wavelength 650 or 620 nm)

● **Calculation of results :**

A calibration curve was constructed by plotting the absorbance values obtained for calibrators on the y-axis against the corresponding NGAL concentrations on the x-axis. The NGAL concentrations of diluted samples were found by placing their absorbance values on the calibration curve and reading the corresponding concentration from the x-axis.

Statistical analysis:

Data were analyzed with SPSS version 20 (statistical package for the Social Science, Chicago, IL). Data are presented as mean ±standard deviation when normally distributed, and as median and interquartile range for skewed data. Differences between patients and controls were tested using Mann–Whitney U or Student's T testing where appropriate. Correlations were performed using Spearman's correlation coefficients. While qualitative data were expressed as number and percentage and were analyzed by Chi square (X2) test. The receiver operating characteristic (ROC) curve and 95% confidence interval (CI) was performed to determine cutoff values for the studied biomarkers. Optimal cutoff points were determined by the highest sum of specificity and sensitivity calculated using receiver-operator curve analysis. Cutoffs for the model were then chosen at clinically relevant or numerically easy cutoff points. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined. P-value was considered significant if < 0.05 and highly significant if < 0.001.

RESULTS

There are a statistically non-significant difference between cases and controls as regards both age and gender (table 1).

Ejection fraction (EF)values, levels of BUN, serum creatinine and NGAL are significantly higher, while eGFR values are significantly lower among chronic HF cases as compared to their controls (table 2).

There is a significant positive correlation between serum NGAL level and creatinine while there is a significant negative correlation with eGFR in chronic HF cases (figures 1 and 2).

Receiver operating characteristic (ROC) curve showed an optimal NGAL cutoff at > 18 μg/L, with sensitivity of 93%, specificity of 76% for diagnosis of renal impairment in chronic HF, with area under curve (AUC=0.912, P=0.001) (figure 3).

Levels of BUN, serum creatinine and NGAL are significantly higher, while eGFR and EF values are significantly lower among acute HF cases as compared to their controls at admission (table 3).

Levels of BUN, serum creatnine and NGAL on the 3rd day are significantly higher than their levels at admission, while eGFR values are significantly lower on the 3rd day as compared with admission day (table 4).

Levels of BUN, serum creatinine and NGAL on admission day are significantly higher, while eGFR values are significantly lower in acute HF cases with WRF than cases without WRF (table 5).

Levels of BUN, serum creatinine and NGAL on day (3) are significantly higher, while eGFR values are significantly lower in acute HF cases with WRF than cases without WRF (table 6).

Levels of BUN, serum creatnine and NGAL on day (3) are significantly higher, while eGFR values are significantly lower on day (3) as compared with admission day in acute HF cases with WRF (table 7).

There are significant positive correlation between admission NGAL level and creatinine and significant negative correlation with eGFR in acute HF cases (figures 4 and 5).

A receiver operating characteristic (ROC) curve showed an optimal admission NGAL cutoff at > 27.5µg/L, with sensitivity of 90% and specificity of 68% for WRF prediction in acute HF, with area under the curve (AUC= 0.969, P=0.001) (figure 6)

Table (1): Demographic data among chronic HF patients, acute HF patients and controls.

Variable	**Chronic HF (N=30)**		**Acute HF (N=30)**		**Controls (N=20)**		**Test of sig**	**P**	**sig**
Age (years): Mean ± SD	48.5 ± 10.1		47.9 ± 11		48.2 ± 10		F = 0.03	0.97	NS
Gender:	No	%	No	%	No	%			
Male	14	46.7	15	50.0	9	45.0	$\chi 2$ = 0.1	0.9	NS
Female	16	53.3	15	50.0	11	55.0			

NS: non-significant.

Table (2): Comparison between chronic HF cases and control group as regards different parameters on admission

Parameter	**Chronic HF (N= 30)**	**Controls (N=20)**	**P**
EF (%) Mean ±SD	34.0±9.1	75.4±4.7	0.000**
BUN (mg/dl) Median (Range)	15 (4 – 36)	7 (3 – 14)	0.002*
S.Creatnine (mg/dl) Median (Range)	0.6 (0.2 - 1.6)	0.3 (0.2 - 0.6)	0.004*
eGFR (ml/min/1.73m^2) Median (Range)	65 (50.3 – 168)	101.2 (84.1 –165)	0.003*
Serum NGAL (µg/L) Median (Range)	13.7 (3 – 55)	4.5 (2 – 8)	0.001**

EF: Ejection Fraction. BUN: Blood Urea Nitrogen. eGFR: Estimated glomerular filtration rate. NGAL: neutrophil gelatinase associated lipocalin. ** high significance. * Significant

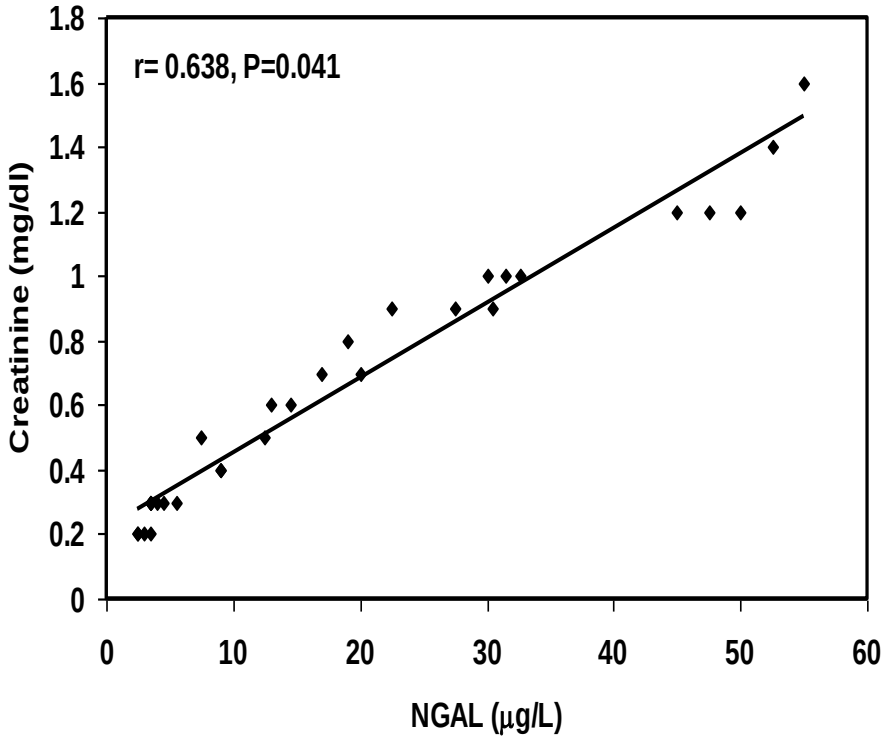


Figure (1): Correlation between levels of serum NGAL and serum creatinine in chronic HF group.

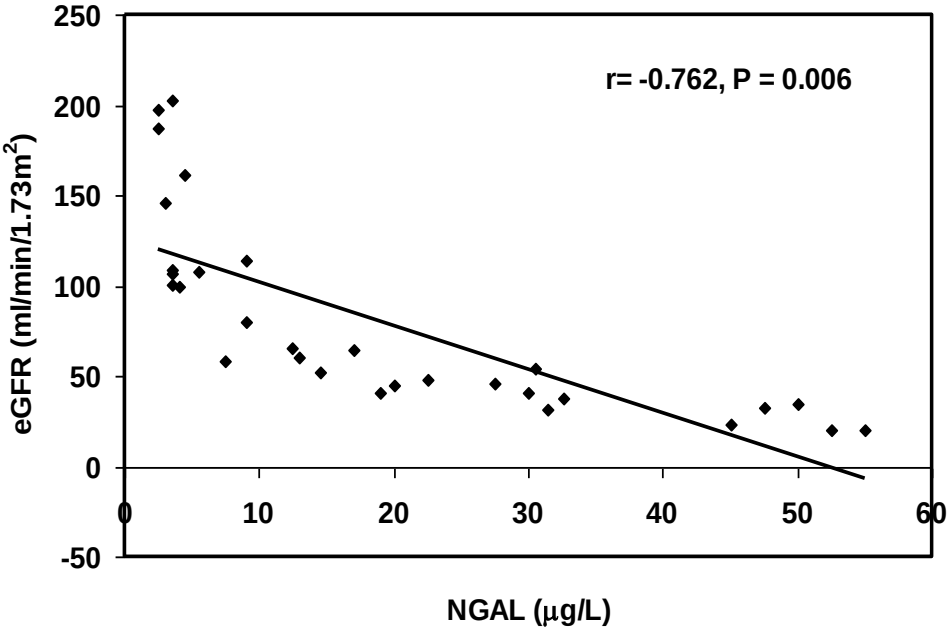
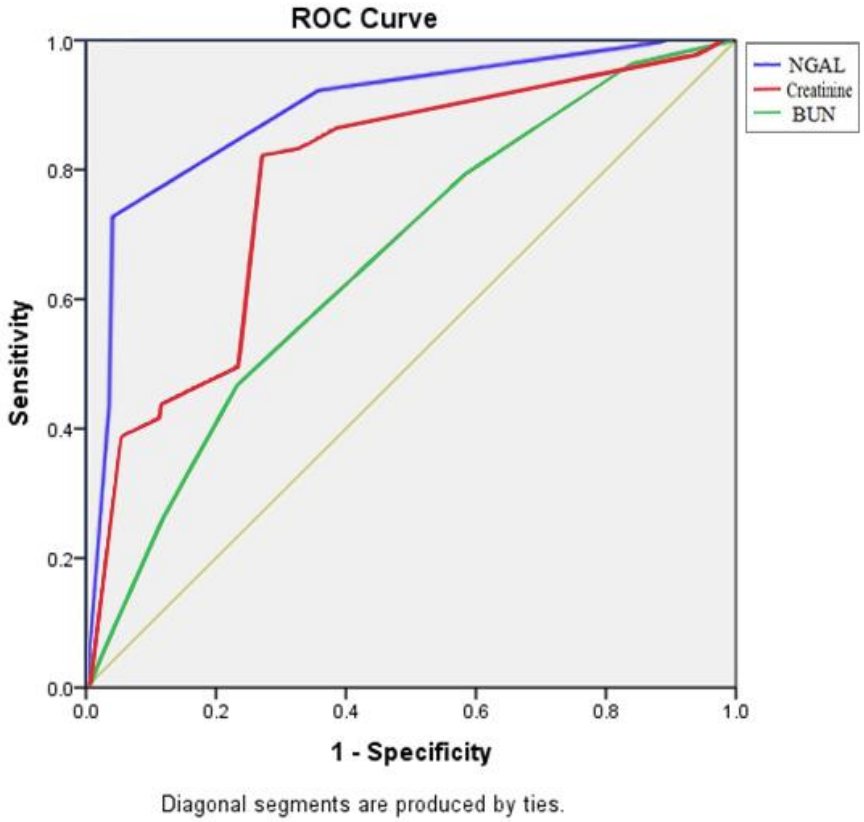


Figure (2): Correlation between NGAL level and eGFR in chronic HF group.



Parameter	AUC	95% CI	P
NGAL	0.912	0.791 – 0.983	0.001**
Creatinine	0.729	0.639 - 0.818	0.001**
BUN	0.625	0.564 - 0.708	0.037*

Figure (3): Receiver operating characteristic (ROC) curve analysis for diagnostic values of serum NGAL, creatinine and BUN for renal impairment in chronic HF cases.

Table (3): Comparison between acute HF cases and control group as regards different parameters on admission day.

Parameter	**Acute HF (N= 30)**	**Controls (N=20)**	**P**
EF (%) Mean±SD	34.9±9.9	75.4±4.7	0.000**
BUN (mg/dl) Median (Range)	15.5 (4-43)	7 (3-14)	0.032*
S.Creatnine (mg/dl) Median (Range)	0.5 (0.2-2.8)	0.3 (0.2-0.6)	0.014*
eGFR (ml/min/1.73m^2) Median (Range)	86.7 (50.6-158)	101.2(84.1 – 165)	0.001**
Serum NGAL (µg/L) Median (Range)	12.5 (3-50)	4.5 (2-8)	0.002*

EF: Ejection Fraction. BUN: Blood Urea Nitrogen. eGFR: Estimated glomerular filtration rate. NGAL: neutrophil gelatinase associated lipocalin. * significant. ** high significance.

Table (4): Comparison between admission (day 1) and third day as regards renal parameters and serum NGAL

in acute HF cases

Parameter	Acute HF (N= 30) Day (1)	AcuteHF (N=30) Day (3)	P
BUN (mg/dl) Median (Range)	15.5 (4-43)	18 (6 – 52)	0.04*
S.Creatnine (mg/dl) Median (Range)	0.5 (0.2-2.8)	0.75 (0.2 – 3.2)	0.001**
eGFR(ml/min/1.73m^2) Median (Range)	86.7(50.6-158)	64.5 (42-140)	0.000**
Serum NGAL (µg/L) Median (Range)	12.5 (3-50)	29.05 (3 – 53)	0.000**

BUN: Blood Urea Nitrogen. eGFR: Estimated glomerular filtration rate.
NGAL: neutrophil gelatinase associated lipocalin. * significance. ** high significance.

Table (5): Comparison between acute HF cases with WRF and cases without WRF as regards renal parameters and serum NGAL at admission (day 1).

Parameter	Acute HF (N=30)		P
	WRF (N=17) (56.6%)	No WRF (N=13) (43.4%)	
BUN (mg/dl) Median (Range)	22 (4 – 43)	8 (4 – 26)	0.023*
S.Creatnine (mg/dl) Median (Range)	0.7 (0.2 – 2.8)	0.5 (0.2 – 1.3)	0.041*
eGFR (ml/min/1.73m^2) Median (Range)	72 (50.6-95)	105 (79.8-158)	0.000**
Serum NGAL (µg/L) Median (Range)	30 (3 – 50)	3.5 (3 – 27)	0.000**

WRF: Worsening renal function. BUN: Blood Urea Nitrogen. eGFR: Estimated glomerular filtration rate.
NGAL: neutrophil gelatinase associated lipocalin. * significance. ** high significance.

Table (6): Comparison between acute HF cases with WRF and cases without WRF as regards renal parameters and serum NGAL on day (3).

Parameter	Acute HF (N=30)		P
	WRF (N=17)(56.6%)	No WRF (N=13)(43.4%)	
BUN (mg/dl) Median (Range)	26 (6 – 52)	11 (6 – 18)	0.000**
Creatnine (mg/dl) Median (Range)	1.1 (0.5 – 3.2)	0.4 (0.2 – 0.5)	0.000**
eGFR (ml/min/1.73m^2) Median (Range)	53.7 (42-75.3)	90 (64-140)	0.000**
Serum NGAL (µg/L) Median (Range)	38 (10-53)	5 (3 – 18)	0.000**

WRF: Worsening renal function.BUN: Blood Urea Nitrogen. eGFR: Estimated glomerular filtration rate.
NGAL: neutrophil gelatinase associated lipocalin. ** high significance.

Table (7): Comparison between day (1) and day (3) renal parameters in acute HF cases with WRF.

Parameter	WRF (N=17) Day (1)	WRF (N=17) Day (3)	P
BUN (mg/dl) Median (Range)	22 (4 – 43)	26 (6 – 52)	0.041*
S.Creatinine (mg/dl) Median (Range)	0.7 (0.2 – 2.8)	1.1 (0.5 – 3.2)	0.001**
eGFR (ml/min/1.73m^2) Median (Range)	72 (50.6-95)	53.7 (42-75.3)	0.001**
Serum NGAL (µg/L) Median (Range)	30 (3 – 50)	38 (10-53)	0.001**

WRF: Worsening renal function. BUN: Blood Urea Nitrogen. eGFR: Estimated glomerular filtration rate. NGAL: neutrophil gelatinase associated lipocalin. * significance. ** high significance.

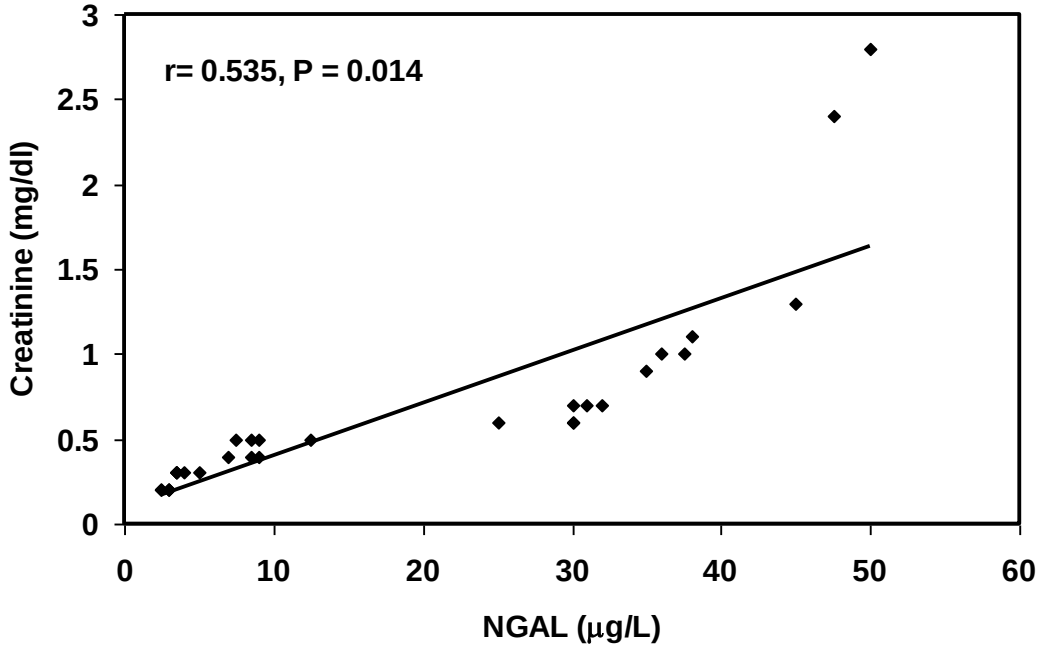


Figure (4): Correlation between admission NGAL level and serum creatinine in acute HF group.

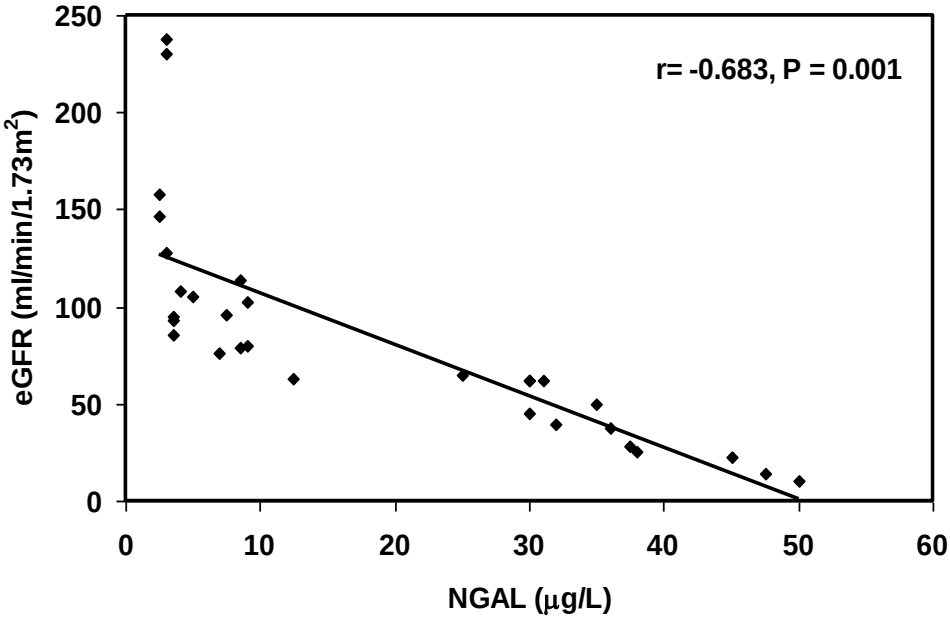
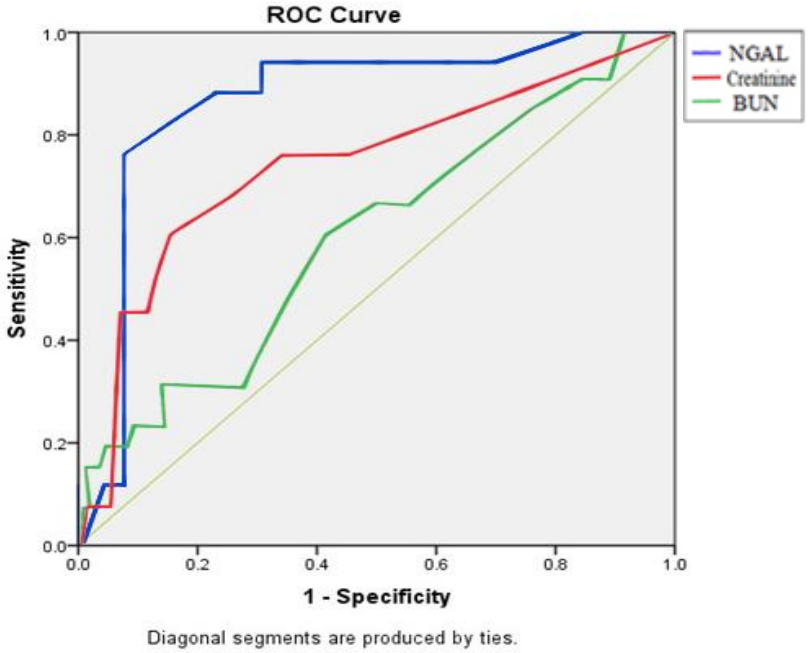


Figure (5): Correlation between admission NGAL level and eGFR in acute HF group.



Parameter	AUC	95% CI	P
NGAL	0.969	0.843 – 1.001	0.001**
Creatinine	0.684	0.508 – 0.711	0.023*
BUN	0.569	0.432 – 0.623	0.632

Figure (6): Receiver operating characteristic (ROC) curve analysis for predictive values of admission NGAL ,serum creatinine and BUN for WRF in acute HF cases.

DISCUSSION

Renal insufficiency is frequently observed in patients with chronic heart failure, these patients have been classified as having CRS type 2. It had an overall prevalence of 57% in an analysis of hospitalized patients with chronic heart failure[11].

Acute Decompensated Heart Failure (ADHF) is frequently associated with deterioration in renal function, acute heart failure patients whose renal function worsens during the acute episode have been classified as having CRS type 1. In a study of patients admitted to hospital with ADHF, 27% had subsequent deterioration of renal function which was associated with increased mortality and length of hospital stay[12, 13].

Based on the important clinical utility of NGAL as a diagnostic and predictive marker for renal tubular injury in adult patients with heart failure, NGAL was an attractive potential biomarker for us to be studied. Therefore, our study aimed to assess the role of serum NGAL in predicting subsequent development of worsening renal function (WRF) in case of ADHF. Also we aimed to assess the role of serum NGAL as a diagnostic marker for renal impairment in chronic heart failure and to establish the relationship between serum NGAL and other renal parameters (BUN, creatinine and eGFR).

Our study included 30 cases with chronic HF, 30 cases with acute HF and control group which included 20 persons, age and sex matched apparently healthy individuals.

All subjects in our study were carefully selected to exclude the confounding factors that influence the level of serum NGAL as recurrent urinary tract infections, end stage renal disease or known exposure to nephrotoxic agents.

Chronic heart failure results in the onset or progression of CKD, clinical studies indicate that extended periods of chronic heart failure result in altered renal hemodynamics followed by progressive renal pathology. Experimental and clinical data indicated that CRS type 2 is characterized by mild to moderate proteinuria, progressive decline of GFR, and an elevated expression of renal injury biomarkers[11].

Important pathophysiological triggers of renal disease progression include chronic increases in renal venous pressure, maladaptive activation of the renin-angiotensin-aldosterone axis and the sympathetic nervous system, as well as a chronic inflammatory state. Yet, clinical interventional trials that directly test the impact of renin-angiotensin system antagonists and β-blockers on the progression of CKD in CRS type 2 are lacking[14].

Echocardiography is a very important tool in diagnosis of CHF, it can confirm enlargement of ventricular chambers and impaired left ventricular systolic functions including EF and fraction shortening (FS)[15].

In patients with chronic HF, renal insufficiency (defined as eGFR < 60 ml/min/1.73 m^2) is common and is associated with severely increased morbidity and mortality. Renal impairment is not only associated with decreased GFR, but also with the presence of structural tubular damage, as measured by increased urinary concentrations of specific tubular marker proteins[9].

In the setting of CKD, NGAL expression may represent active tubular damage beyond a marker of decreased glomerular filtration alone and predicts disease progression[16].

In the present study, eGFR values were significantly lower while BUN, serum creatinine and NGAL levels were significantly higher in chronic HF cases as compared with their controls. This result is in agreement with **Damman et al.[17], Damman et al.[18], Shrestha et al.[19] and Nymo et al.[20].**

Although median admission eGFR values in our chronic HF cases appeared to be normal (65 [IQR 50.3 – 168] ml/min/1.73m^2), serum NGAL levels were significantly increased, this finding suggests that although glomerular function and integrity seem normal, subclinical tubulointerstitial damage exists in some patients. This situation may exist when the kidney is already compensating for a loss in nephrons and therefore an unnoticed absolute loss in total GFR in heart failure. Elevated serum NGAL level may therefore indicate early impairment of GFR even before eGFR itself is affected[21].

Blood and urine NGAL levels generally correlate with clinical and biochemical markers of heart failure severity [14].

In chronic HF, reduced GFR is mainly dependent on reduced renal perfusion, which may serve as a hypoxic trigger for tubular damage. Chronic renal hypoxia has not only been proposed as the final common pathway to end

stage renal disease, but may also be the initiating trigger for a vicious circle between tubulointerstitial injury and chronic renal insufficiency. This hypothesis may be one of the pathways by which chronic renal insufficiency may develop in patients with chronic HF[11].

There are different histopathological pathways of tubulointerstitial injury induced by glomerular damage. This damage is associated with hypoxia and oxidative stress on a tubular level, and eventually nephron loss, which, in turn, impose hemodynamic stress and damage in the remaining nephron units. Therefore, a sensitive marker of tubular injury, which can be used to identify or confirm the presence of glomerular injury would be helpful in the evaluation of the time course of renal function and renal damage in CHF patients[21]. So, we examined the relation of serum NGAL, as a marker of renal inflammation with other parameters of renal function, including serum BUN, creatinine and eGFR.

In the present study, serum NGAL level had significant positive correlations with BUN and serum creatinine, while it had significant negative correlation with eGFR. This result runs in parallel with observations reported by **Damman et al.[17], Damman et al.[18], Nymo et al.[20] and Mitsnefes et al.[22]** who found that both serum and urinary NGAL levels had significant positive correlations with serum creatinine level and significant negative correlations with eGFR in chronic HF cases.

Although all forms of CKD are associated with tubulointerstitial injury, regardless of whether the primary pathology is glomerular or otherwise, it is widely accepted that in some CKD, the rate of deterioration in renal function and the overall outcome are more accurately associated with the degree of renal tubulointerstitial damage rather than with the severity of glomerular lesions. These factors suggest that NGAL may have unique predictive value in progression of renal injury in CRS type 2[23].

A ROC curve analysis was performed to evaluate the individual diagnostic values of NGAL, creatinine and BUN for renal impairment in our chronic HF cases. With adjusting eGFR as gold standard, an optimal NGAL cutoff at > 18 μg/L, yielding sensitivity of 93% and specificity of 76% with area under curve (AUC) of 0.912. While BUN and serum creatinine had AUC of 0.625 and 0.729 respectively. Therefore, NGAL can be considered as a sensitive diagnostic marker superior to both BUN and creatinine for early detection of impaired renal function in chronic HF cases even before eGFR is markedly reduced.

In patients with acute HF, renal insufficiency is frequent, the early identification of such patients may represent an opportunity to develop strategies aiming for the preservation of kidney function. For example, careful titration of loop diuretic doses and avoidance of potential nephrotoxins such as intravenous radiographic contrast media[24].

In our study, acute HF group included 15 (50%) males and 15 (50%) females. Samples for NGAL assay were obtained at enrollment (admission) and on day (3) while changes in BUN, serum creatinine and eGFR were monitored during their hospital stay for 3 days to assess WRF.

NGAL has emerged as a promising and sensitive biomarker of early AKI in a diverse range of settings, as NGAL rapidly appears in both blood and urine within 24 to 48 hours before plasma creatinine increase[16].

Our study demonstrated that BUN, serum creatinine and serum NGAL levels on admission were significantly higher, while admission eGFR values were significantly lower in acute HF cases as compared with their controls. This result is parallel to the observations demonstrated by **Aghel et al.[25] and Shrestha et al.[26].**

The follow up of levels of BUN, serum creatnine and NGAL on day (3) showed that they were significantly increased as compared with admission day, while eGFR values were significantly decreased on day (3) as compared with admission day. This result is in agreement with **Aghel et al.[25] and Alvelos et al.[27].**

WRF (defined as increase in serum creatinine (S.Cr) ≥0.3 mg/dL (≥26.5 μmol/L) or ≥25% relative to S.Cr at the time of hospital admission) is common in patients with acute heart failure and is associated with significant early and late morbidity and mortality[3]. Our study showed that, 17 (56.6%) patients developed WRF within the 3days hospital follow up and 13 (43.4%) patients didn't develop WRF.

Traditional explanations regarding the mechanisms of WRF in the setting of ADHF include overzealous diuresis, leading to reduced renal perfusion, and low cardiac output heart failure resulting in acute tubular injury. These can lead to excessive neurohormonal activation and altered tubuloglomerular feedback. In addition, more studies have demonstrated the association between venous congestion rather than low cardiac output with WRF in ADHF. Clearly, no single mechanism can explain this complex pathophysiologic interaction between the failing heart and the impaired kidneys[12].

Due to the high incidence and poor prognosis associated with WRF in the setting of acute heart failure, earlier reports have highlighted a role for NGAL in predicting WRF in the setting of ADHF[9].

Our results detected that, BUN, serum creatinine and NGAL levels on admission were significantly higher, while admission eGFR values were significantly lower in acute HF cases with WRF than their levels in cases without WRF. This result is in agreement with **Alvelos et al.**[27].

Aghel et al.[25] investigated 91 patients with ADHF, 38% developed WRF within the 5-day hospital follow-up. Patients who developed WRF versus those without WRF had significantly higher admission serum NGAL and creatinine levels and significantly lower eGFR values, and this support our result. Similarly, **Macdonald et al.**[12] **and Breidthardt et al.**[28] had observed that all renal parameters including BUN, serum creatinine and NGAL were significantly higher, while eGFR values were significantly lower in ADHF cases with AKI as compared with cases without.

On the contrary, **Dupont et al.**[29] found that, baseline urinary NGAL levels, in patients with ADHF, were not different among patients who developed WRF and those who didn't.

Also, we observed that BUN, serum creatinine and NGAL levels on day (3) were significantly higher, while eGFR values were significantly lower in acute HF cases with WRF as compared to those without. This observation is similar to that reported by **Aghel et al.**[25], **Alvelos et al.**[27] **and Dupont et al.**[29] who demonstrated that day (3) renal parameter levels were significantly higher in ADHF cases with WRF as compared to those without.

Although admission serum creatinine levels were higher in acute HF cases with WRF than those without. The shortcoming of creatinine for monitoring renal function in the acute setting is well known. Unfortunately, serum creatinine is a delayed and unreliable indicator of AKI for a variety of reasons; serum creatinine concentrations do not reflect the true decrease in GFR in the acute setting, since several hours or days must elapse before a new equilibrium between the presumably steady-state production and the decreased excretion of creatinine is established. In addition, serum creatinine is influenced by several non renal factors. Also, a number of acute and chronic kidney conditions can exist with no increase in serum creatinine owing to the concept of renal reserve, it is estimated that over 50% of kidney function must be lost before serum creatinine rises[30].

On follow up, there were no significant changes regarding renal parameters (BUN, creatinine, eGFR) and NGAL during 3-day hospital stay in acute HF cases without WRF. But it was found that, in acute HF cases with WRF, BUN, serum creatinine and NGAL levels were significantly higher while eGFR values were significantly lower on day (3) as compared with admission day.

We reported that, NGAL level had significant positive correlations with BUN and serum creatinine, while it had significant negative correlation with eGFR on admission. These results are consistent with **Aghel et al.**[25], **Shrestha et al.**[26], **Alvelos et al.**[27], **Bredithardt et al.**[28] **and Maisel et al.**[31].

A ROC curve analysis was performed to evaluate predictive values of of admission NGAL, serum creatinine, eGFR and BUN for WRF in acute HF, with adjusting eGFR as a gold standard, an optimal admission NGAL cutoff at > 27.5µg/L yielding 90% sensitivity and 68% specificity with AUC 0.969. While BUN and serum creatinine had AUC of 0.569 and 0.684 respectively.

Although acute HF cases with WRF had higher levels of BUN and lower eGFR values than those without WRF on admission, the performance of these renal parameters was non-satisfactory as expressed by the AUC. Therefore, NGAL can be considered as a sensitive marker superior to both BUN and eGFR for prediction of WRF in acute HF even before serum creatinine is significantly affected. This suggests that ongoing or prior renal tubular injury detected by elevated admission NGAL measurements is the "driving factor" for WRF during hospitalization[11].

The association of higher serum NGAL levels with WRF was already demonstrated by **Aghel et al.**[25] who found that, by ROC curve analysis, admission NGAL ≥140 ng/mL had a sensitivity and specificity of 86% and 54% respectively, to predict the development of WRF in ADHF patients. The AUC for admission NGAL, eGFR and BUN were 0.70, 0.61, 0.56 respectively.

Alvelos et al.[27] found that the AUC for NGAL in predicting CRS type1 in ADHF patients was 0.93. The best cutoff value (170 ng/ml) had a sensitivity of 100% and a specificity of 86.7%.

Macdonald et al.[12] had reported that, for prediction of AKI in patients with ADHF, admission NGAL > 89 ng/ml had sensitivity of 68% and specificity of 70% with AUC of 0.71. Also, **Shrestha et al.**[26] considered both serum and urine NGAL as sensitive markers for AKI prediction with AUC of 0.67 and 0.64 respectively.

Haase et al.[13] presented a meta-analysis consisting of 1217 patients of ADHF, with clinical outcomes of AKI patients according to NGAL and creatinine status. A major finding was that 41% of patients diagnosed with AKI would have been missed by creatinine alone. While NGAL tended to predict AKI risk even when creatinine was not significantly changed.

Maisel et al.[31] reported that the main finding of his study is that plasma NGAL at the time of hospital discharge is a powerful predictor of 30 days outcome in patients with ADHF. It is substantially superior to conventional measures of renal function such as BUN, serum creatinine and eGFR. Thus, NGAL is not only a risk predictor for renal injury but is an overall strong risk marker for cardiac events in the setting of ADHF.

In contrast, **Breidthardt et al.**[28] found that plasma NGAL level couldn't adequately predict neither WRF nor AKI in patients with ADHF with AUC of 0.52. As discussed earlier, studies have reported different cutoff levels for NGAL to predict WRF, probably because of the heterogeneity among studies. Therefore, it seems that each clinical setting requires the determination of a normal range and cutoff value.

Finally, all our results corroborate prior reports demonstrating the relationship between elevated systemic NGAL levels and renal insufficiency, and support the utility of systemic NGAL for detecting underlying renal vulnerability that may predict subsequent WRF in the ADHF setting .

However, the present study has some limitations, as it was a single-center study, and the sample size may be considered relatively small, which restricts the power of conclusions.

CONCLUSION

NGAL can be considered as a sensitive diagnostic marker superior to both BUN and creatinine for early detection of impaired renal function in chronic HF even before eGFR is markedly reduced.

Also, NGAL can be considered as a sensitive marker superior to both BUN and eGFR for prediction of WRF in acute HF even before serum creatinine is significantly affected. This important finding suggests that early management strategies may have a significant impact on subsequent WRF and outcomes.

Future studies should consider a role for NGAL in guiding therapeutic or disposition decisions that could alter the course of WRF and improve outcomes.

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